Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Antifertility effect of chronically administered *Tabernaemontana divaricata* leaf extract on male rats

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ARTICLE INFO

Article history:
Received 22 December 2011
Received in revised form 15 April 2012
Accepted 18 July 2012
Available online 20 July 2012

Keywords:
Tabernaemontana divaricata
Anti-fertility
Male rats
50% ethanol extract
Reproduction
Hormone levels

ABSTRACT

Objective: To investigate the antifertility effect of chronically administered *Tabernaemontana divaricata (T. divaricata)* leaf extract on male rats. **Methods:** The effect of 50% ethanol extract of *T. divaricata* leaves on reproduction was studied on male rats. The study was divided into four groups of five animals each. The first groups (I) received vehicle alone to serve as control. The second, third and fourth groups (II, II and IV) of animals were administered the leaf extract daily at 50 mg/kg body weight, *p.o.*,100 mg/kg body weight, *p.o.*, and 200 mg/kg body weight, *p.o.*, respectively, for a period of 60 days. **Results:** Significant decreases in the weight of testes, epididymis, seminal vesicle and ventral prostate were observed. A dose related reduction in the testicular sperm count, epididymal sperm count and motility, number of fertile male, ratio between delivered and inseminated females and numbers of pups were observed. The testis showed a clear correlation between the dose and severity of lesions of seminiferous epithelium. In general, the seminiferous tubules appear reduced in size with a frequently filled eosinophilic material. Spermatogenesis arrested at the secondary spermatocyte stage. Pachytene spermatocytes were undergoing degeneration. Disorganigation and sloughing of immature germ cell were visible. Leydinf cells were atrophied. No morphological changes were observed in Sertoli cells. Significant reduction in serum concentration of luteinizing hormone and testosterone were observed. No distinct change in serum FSH concentration was recorded. The final body weights of all groups were elevated markedly. No alterations were recorded in any hematologiocal parameters. **Conclusions:** It is concluded that the 50% ethanol extract of *T. divaricata* leaf produced dose related effect on male reproduction without altering general body metabolism.

1. Introduction

The quest for the oral contraceptive agent that can control human fertility is as old as recorded history. Although a wide variety of synthetic contraceptive agents[1] are available, these cannot be used continuously due to their severe side effects[2, 3]. Hence people are looking back to age old tradition of using herbal medicines, which have minimum side effects. India in general and Western Ghat region in particular has enormous wealth of medicinal plants. Presently, a major programme on systematic investigation of medicinal plants for their phytochemical, biological and pharmacological properties, including antifertility properties, was undertaken in our laboratory[8]. As part of this research programme, we present in this paper antifertility efficacy of leaves of the plant *Tabernaemontana divaricata (T. divaricata)*. A glabrous, evergreen shrub 1.8-2.4 m in height with silvery grey bark and milky latex. Leaves are simple, opposite, elliptic or elliptic-lanceolate, smooth, glossy green, acuminate and wavy margins; flowers are white, sweetly fragrant in 1-8 flowered cymes at the bifurcations of the branches, lobes of corolla overlapping to right in the bud[16]. It is used as thermogenic, anodyne, astringent, vermifuge, odonalgia and in treatment of strangury, paralysis, arthralgia and melalgia. Flower juice mixed with oil alleviates burning sensation, cures eye sore and skin diseases, leaves juice applied to wounds to prevent inflammation and used in opthalmia,[3]. Flower contains Dregamine, 20-epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones,

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flavonol glycosides; isovoacristine, voaphylline-hydroxyindolenine, janetine (tetrahydrolivadine), N-methyl-voaphylline (hecubine). Kaempferol, and leaves contains Dregamine, 20-epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones, flavonol glycosides, isovoacristine, α-amyrin, lupeol and their acetates, β-sitosterol, coronaridine, apparicine, ervaticine (2-acyl indole derivative), ervatinine, hyderabadine, lahoricine, mehranine, stapfinine, voacristine, voharine and a dimeric alkaloid, conophylline 17-β oestrdiol. A literature survey reveals that no systematic approach has been made to study the antifertility activity of leaves of this plant. Therefore the present preliminary investigation reports the reproduction effect of 50% ethanol extract of *T. divaricata* leaves on male rats.

2. Materials and methods

2.1. Animal model

Twenty colony bred adult male albino rats (*Ractus norvegicus*, Sprague - Dawley strain), 3-5 months old and weighing between 175 and 250 g with proved fertility were marked properly and housed two or three animals in polypropylene cages under controlled environmental conditions (12- h light: 12- h dark). They were fed pelleted standard rat feed (Ashirwad Food, Chandigarh, India) supplemented with soaked gram and wheat and allowed free access to water.

2.2. Plant material

One collection of the *T. divaricata* leaves from Indore, Madhya Pradesh India. The leaves were shade dried, powered and soxhlated with ethanol (50% v/v) at 55-60 ℃ for 36 h. the solvent was distilled off under petroleum ether, benzene, chloroform and acetone to remove impurities left, if any during extraction. Thus the resulting mass was dried under vacuum and kept at 4 ℃.

2.3. Treatment protocol

Animals were equally distributed into four treatment groups containing five in each.

Group I—Animals in this group were given vehicle (Sterile distilled water) alone *p.o.* for 60 days to serve as vehicle treated control; Group II—Animals in this group were treated with *T. divaricata* leaf (50% EtOH) extract at the dose of 50 mg/kg body weight/day; *p.o.* for 60 days; Groups III—Animals in this groups received *T. divaricata* leaf (50% EtOH) extract at the dose of 100 mg/kg body weight/day; *p.o.* for 60 days; Group IV-- Animals in this groups received *T. divaricata* leaf (50% EtOH) extract at the dose of 200 mg/kg body weight/day; *p.o.* for 60 days.

A suspension of the extract was prepared in sterile distilled water (100 mg/mL) before administration. The required drug was administered orally with a glass syringe fitted with a feeding needle.

2.4. Sacrification schedule

Twenty-four hours after their last dose, the rats were weighed and sacrificed under light ether anesthesia.

2.5. Parameter

2.5.1 Sperm motility and count

Cauda epidymal sperm motility and count, and testicular sperm count (maturing spermatozoa with head and tail) were made[12]. One hundred milligram of each tissue was minced in 1 mL of physiological saline. For sperm motility, one drop of evenly mixed sample was applied to a glass slide under the cover glass. The percent motility was determined by counting both motile and immotile spermatozoa per unit area epididymal and testicular sperm count were also made expressed as million/mm^3 of suspension.

2.5.2. Fertility test

Male rats were introduced to female, 200-300 g (male: female ratio, 1:2) at 17:00 h after 55 days of treatment. The successful mating was confirmed in the forthcoming morning from 56 to 61 day by vaginal plug and spermatozoa in the vaginal smear. The inseminated female were separated and allowed to deliver at term, and the number of pups delivered and their characteristics were noted.

2.5.3. Body and organ weights

The initial and final body weights of the animals were recorded. The testes, epididymides, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram.

2.5.4. Testicular histology

One (right) of the two testes of each animal was fixed in Bouin's fixative solution, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. Section were cut at 5 micrometer, stained with Harris hematoxylin and eosin and observed under a light microscope.

2.5.5. Radioimmunoassay of hormones

Blood sample were also collected for estimations of serum gonadotropins and testosterone by radioimmunoassay (RIA). Serum sample were separated by standard procedure and stored at - 20 ℃ for subsequent analysis.

2.5.6. Hematology

The counts of RBC and WBC, hemoglobin, hematocrit,

and standard hematological indices (color index, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and mean corpuscular volume) were determined from the blood collected directly from the heart at the time of scarification[9].

2.6. Ethical aspects

The study was approved by the Institutional animal ethical committee (IAEC) of the College of Pharmacy IPS Academy, Indore MP India

2.7. Statistical analysis

Data are expressed as mean ± S.D. and analyzed for statistical significance by using one way analysis of variance (ANOVA). Results were considered significant at the $P \leqslant 0.05$ level.

3. Results

3.1. Sperm motility and count

A significant decrease ($P \leqslant 0.01$) in percent cauda epididymal sperm motility was evident in groups II, III and IV animals when compared with group I animals. After 60 days of treatment only (37.5 ± 9.8)%, (32.5 ± 6.7)% and (29.4 ± 9.8)%, respectively, of spermatozoa versus (60.2 ± 2.5)% of spermatozoa in group I was found to be motile. The sperm count from the cauda epididymis and testis were also diminished significantly in all treatment groups ($P \leqslant 0.01$) (Table 1).

3.2. Fertility

The number of fertile males decreased in all treatment groups, leaving 3, 2 and 1 still fertile 60 days of treatment, respectively, in groups II, III and IV. The ratio between delivered and inseminated female (5/10, 4/10 and 2/10 animals versus 10/10 animals in groups I), and the number of pups (50, 28 and 16 pups versus 90 pups in group I) dropped after 60 days of treatment. However, no significant difference was observed in the litter size of the female in any group. Spermatozoa with shortened and thinned flagella were present in the semen found in the vaginal smears of females, which were cohabited with the treated males. All delivered pups were normal healthy (Table 2).

3.3. Body and organ weights

The final body weight of all groups increased markedly when compared with their respective initial body weights ($P \leqslant 0.01$). The final weights of group III (100 mg/kg b.wt., *p.o.*) and group IV (200 mg/kg b.wt., *p.o.*) significantly increased when compared with the final body weight of group I (vehicle treated control) animals ($P \leqslant 0.01$). A great decline in the weights of testes, epididymides, seminal vesicle and ventral prostate (expressed in mg/100 g of body weight) were observed in all treatment groups when compared with group I animals (Table 3).

3.4. Histopathology of testis

The testes of group I (vehicle treated control) animals showed normal features with successive stage of transformation of the seminiferous epithelium into spermatozoa. Leydig cells were situated in between the tubules (Figure 1). Histopathological examination of the testis after 60 days of treatment showed a clear correlation between the dose and the severity of lesion of the seminiferous epithelium. In rats treated with 50 mg/kg, *p.o.* (group II) some lesion were observed and affected only a few tubules (Figure 2). The dose of 100 mg/kg, *p.o.* (group III) produced diffuse changes of the tubules (Figure 3). In rates treated with 200 mg/kg, *p.o.* (group IV) almost all tubules were affected (Figure 4). Since the differences among the doses were more quantitative than qualitative, only a general description of the findings related to all treatment groups is given. The seminiferous tubules to appear reduced in size with a frequently filled eosinophilic material but with normal lamina propria. In general, diminished spermatogenesis was evident at secondary spermatocyte stage. Pachytene spermatocytes were undergoing degeneration. Disorganization and sloughing of immature germ cells were visible. The nuclei became pyknotic. Leydig cells revealed sign of atrophy. Contrary to this, no morphological changes were observed in the Sertoli cells.

Table 1
Sperm characteristic after 60 days of treatment with 50% ethanol extract of *T. divaricata* leaves on male rats.

S. No.	Treatment group	Sperm motility count (%) Cauda epididymis	Sperm count (million/mm^3)	
			Testis	Cauda epididymis
1	Group I	60.2±2.8	4.8±0.2	50.0±3.2
2	Group II	37.5±9.8*	2.6±0.8*	20.2±7.0*
3	Group III	32.5±6.7*	2.1±0.5*	16.0±1.5*
4	Group IV	29.4±9.8*	1.9±0.4*	11.0±2.4*

Data are expressed as mean±S.D., $n = 5$, *$P < 0.01$ Compared with corresponding group I.

Table 2

Fertility of male rats after 60 days of treatment with 50% ethanol ectract of *T. divaricata* leaf (Male: Female ratio, 1: 2.).

S. No.	Treatment groups	No. of fertile males/no. of treated males	No. of females delivered/ no. of inseminated female	Total no. of pops	Litter size±S.D.
1	Group I	5/5	10/10	90	8.50±0.80
2	Group II	3/5	5/10	50	10.60±1.05
3	Group III	2/5	4/10	28	6.50±1.10
4	Group IV	1/5	2/10	16	7.00±1.20

n = 5 (male), n = 10 (female), compared with group I

Table 3

Body and organ weights after 60 days of treatment with 50% ethanol extract of *T. divaricata* leaf on male rats.

S. No.	Treatment groups	Body weight (gm)		Testes [a]	Epididymis [a]	Ventral Prostate [a]	Seminal vesicle [a]
		Initial	Final				
1	Group I	215.0±10.2	285.2±16.0 [b]	1 310.0±16.0	447.4±9.4	270.2±86.0	391.2±6.8
2	Group II	214.0±34.2	283.2±33.3 [b]	908.3±105.6 [c]	333.8±11.8 [c]	122.2±8.2 [c]	328.5±77.4 [c]
3	Group III	210.0±12.5	335.5±46.6 [b,c]	800.2±150.4 [c]	308.5±180.0 [c]	111.8±7.9 [c]	306.8±193.0 [c]
4	Group IV	226.0±16.2	326.0±21.8 [b,c]	772.4±104.9 [c]	297.4±11.4 [c]	110.0±7.9 [c]	285.5±112.7 [c]

Data are expressed as mean±S.D.,[a] mg/100 g of body weight, [b]$P < 0.01$ compared with corresponding initial body weight, [c]$P < 0.01$ compared with corresponding group I.

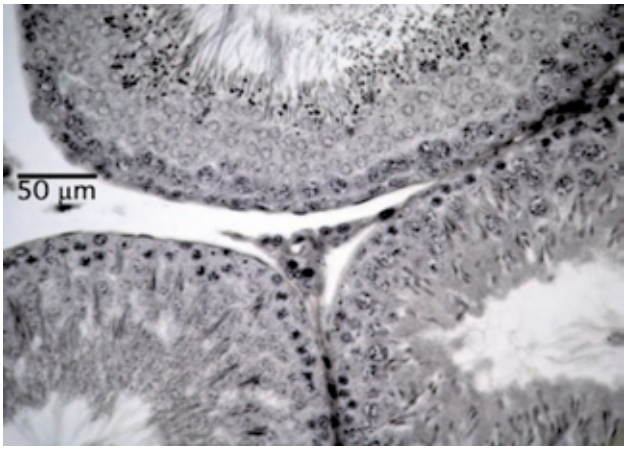

Figure 1. Photomicrograph of testis of a rat of group I (vehicle treated control) showing normal features with successive stage of transformation of seminiferous epithelium to spermatozoa.

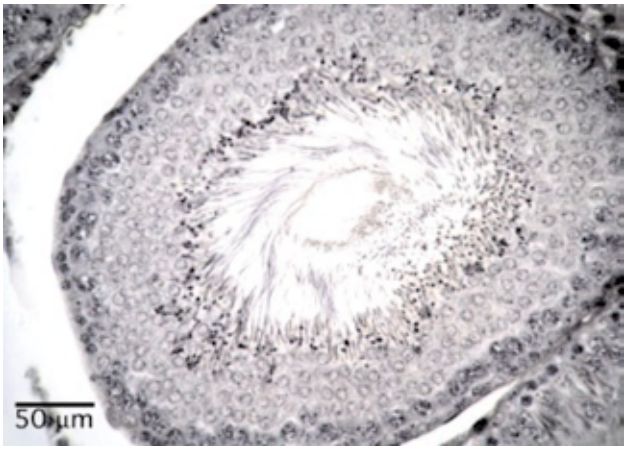

Figure 3. Phomicrograph of testis of a rat of group III (100 mg/kg body weight, *p.o.*) after 60 days of treatment showing cellular damage in tubular elements.

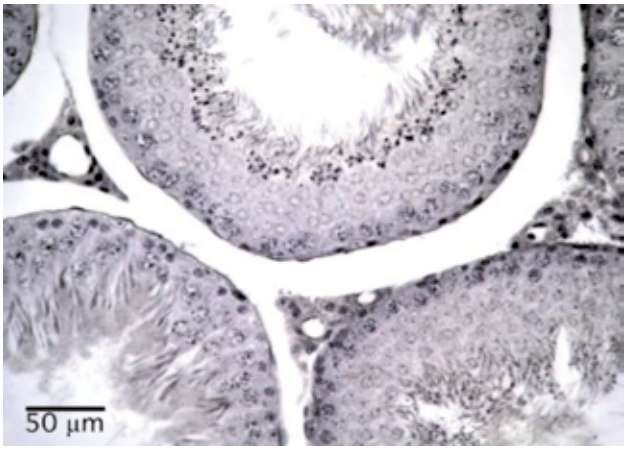

Figure 2. Photomicrograph of an affected region of testis of a rat of group II (50 mg/kg body weight, *p.o.*) after 60 days of treatment. None disorganized germinal epithelium.

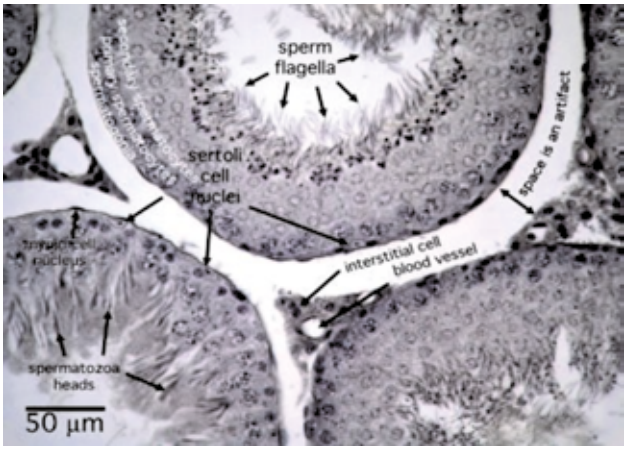

Figure 4. Phomicrograph of testis of a rat of group IV (200 mg/kg body weight, *p.o.*) after 60 days of treatment. Note the arrest of spermatogenesis.

3.5. Heamatology

Hematological parameter, RBC and WBC counts, hemoglobin, hematocrit and standard hematological indices varied within the control range. No drug-related effect on any of these parameters were observed in any groups when compared with vehicle treated control group (group I).

4. Discussion

The present data shows that the 50% ethanol extract of *T. divaricata* leaf suppresses testicular and epididymal sperm counts and causes lesions on the seminiferous tubules related to the dose[10]. The treatment also reduced the serum concentration of LH and testosterone after 60 days of treatment. However, the FSH levels remained unaltered. It is a well known and widely accepted concept that LH is basically responsible for testosterone production[7]. It is probable that the primary step in the mechanism of the effect on testis induced by the *T. divaricata* extract was the suppression of LH. At the testicular level, the absence of stimulation by LH would secondarily cause Leydig cell dysfunction, thereby resulting in decline of testosterone secretion which is responsible for diminished spermatogenesis and hense, reduction in sperm count[4, 11, 13-15].

It is known that the structure and function of the apididymis are dependent on androgens[5]. In the present investigation, a dose related suppression of cauda epididymal sperm motility in all treatment groups suggest an undersupply of testodterone to epididymis and therefore, an impaired epididymal function. The impaired epididymal function may also be due to reduced activity of the testis which affects the normal passage of testicular fluid into the epididymis[9]. This is also confirmed by reduced epididymal weight.

It is well established that hematological testes form the very front-line investigation on which diagnosis of various diseases is based. A significant increase in the final body weight and unaltered hematological parameter in any of the treatment groups in the present investigation in rats, suggest that the *T. divaricata* leaf extract does not cause any adverse effect on general health of the animals.

In conclusion, the oral administration of 50% ethanol extract of *T. divaricata* leaf to male rats produced dose related effect on reproduction. The effect may have an inhibitory influence on gonadrotrophin release which may be responsible for the decline in testosterone production, leading to change in spermatogenesis. Further long term studies are in progress for the evaluation of complete and reversible fertility with this extract and also other extracts of this important plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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